

DOCKET NO. HARR0032-101

PATENT

APPLICATION SERIAL NO. 10/607,479

AMENDMENT AND REQUEST FOR RECONSIDERATION DATED JAN. 6, 2006

REPLY TO OFFICE ACTION OF JULY 6, 2005

IN THE SPECIFICATION

Please amend the specification as follows.

Please replace the paragraph on page 1, beginning on line 11, with the following.

This application is a continuation application of U.S. Application Serial No. 09/798,128, filed March 2, 2001, now U.S. Pat. No. 6,608,037, which claims priority under 35 U.S.C. § 119(a)-(d) of United Kingdom Application No. 0005099.7, filed March 2, 2000 and also claims the benefit under 35 U.S.C. § 119(e) of U.S. Application Serial No. 60/187,465, filed March 6, 2000, and claims benefit under 35 U.S.C. § 119(a)-(d) of PCT Application No. PCT/GB01/00856, filed March 1, 2001, all applications incorporated herein by reference in their entireties.

Please replace the paragraph beginning on line 15 of page 15 with the following.

Figures 1a - c show the results of transient transfections of HeLa, HepG2 and SW480 cells with the Tcf responsive luciferase reporter construct “5merTcf-SV40–Luc” (CTL501) (Figure 1a). The numbers indicate the numbers of base pairs between the Tcf sites. The nucleotide sequence of 5merTCF-SV40 antisense strand (SEQ ID NO: 20) is shown in Figure 1c. The sequences underlined and in italics are active TCF sites. The sequence just underlined is a mutated TCF site. The sequences in bold are the *Bgl*II, *Nhe*I and *Kpn*I recognition sites. Cells were transfected with equimolar amounts (about 1 μ g each) of the Tcf-responsive and control luciferase reporter constructs as indicated in Figure 1b. “SV40p” contains the SV40 promoter only; “SV40 e/p” contains both the SV40 promoter and enhancer; “CMV” contains the cytomegalovirus enhancer/promoter. Data are expressed as fold activation with respect to the activity of the SV40 promoter set as 1. The mean value and SD from two independent transfections are shown. This result is representative of three independent experiments.

Please replace the paragraph beginning on line 8 of page 17 with the following.

Figures 6a-c show the results of transient transfections of HeLa and SW480 cells with the Tcf responsive luciferase reporter construct 5merTcf-E1BTATA-Luc (CTL502) (Fig 6a). The nucleotide sequence of E1BTATA antisense strand (SEQ ID NO:21) is shown in Figure 6c. The sequence underlined and in italics is the E1B TATA box. The sequences in bold are the *HindIII*, *BglIII*, *NheI* and *KpnI* recognition sites. Cells were transfected with equimolar (about 1 μ g each) amounts of several luciferase reporter constructs as indicated in Figure 6b. pGL3basic contains a promoterless luc cDNA; “E1B” contains the Ad5 E1BTATA box upstream of the luc cDNA; 5merTcf-SV40 (CTL501) is described in Figure 1a. Data are expressed as fold activation compared to the activity of pGL3basic set as 1. The mean value and SD of duplicate transfections are shown. The data are representative of three independent experiments.

Please replace the paragraph beginning on line 13 of page 18 with the following.

Figures 9a-c shows the results of transient transfections of SW480 cells using Tcf-E1BTATA luciferase reporter constructs with different numbers and arrangements of Tcf binding sites. Figure 9a shows the number of Tcf sites and the spacing between them. Figure 9c shows the nucleotide sequence of the antisense strands of the Tcf-E1BTATA constructs (SEQ ID NOs: 22, 23, 24, 25, and 26). The sequences underlined and in italics are active Tcf sites. The sequences in bold are the *BglIII*, *NheI* and *KpnI* recognition sites. In the 4merTcf-E1BTATA construct the *BglIII* site is defective. Cells were transfected with 0.5 μ g of each luciferase reporter construct. Data are expressed as percentage activity of 5merTcf-E1BTATA (CTL502) (Fig 9b). The mean value and SD of triplicate transfections are shown. The results are representative of three independent experiments.

Please replace the paragraph beginning on line 26 of page 18 with the following.

Figures 10a-c shows the results of transient transfections of SW480 cells with TcfC-E1BTATA-luc constructs with variable spacing between the proximal Tcf site and the TATA box (25-499 bp). The structure of the TcfC element is described in Figure 7. Figure 10a shows the structure of the TcfC-E1BTATA reporter constructs used for this assay. "d" indicates the number of base pairs from the last nucleotide of the proximal Tcf binding site to the first T of the TATA sequence. The 88 bp and 447 bp spacer fragments were derived by PCR from the human β -globin gene intron II. Cells were transfected with 0.5 μ g of each luciferase reporter construct. Data are expressed as percentage activity of the TcfC-E1BTATA d=25 construct (b). The mean value and SD of triplicate transfections are shown. The results are representative of three independent experiments. The nucleotide sequence of the antisense strand of TcfC-E1BTATA when d=25 (SEQ ID NO: 27) is shown in Figure 10c. The sequences underlined and in italics are active Tcf sites. The sequences in bold are the E1B TATA box, and the *Sma*I and *Kpn*I recognition sites.

Please replace the paragraph beginning on line 30 of page 28 with the following.

pTX0374 was constructed by cloning a 1.6kb *Bgl*II-*Bam*HI fragment containing the CMV promoter fused to the *E. coli* ntr gene into pSW107. This plasmid was constructed by cloning a 917bp fragment of the human beta-globin gene (*Bam*HI site in exon2 to the *Eco*RI site in exon3) coupled to a 240bp *Hinc*II-*Bam*HI fragment containing the polyA addition and transcriptional termination signals of the human complement C2 gene into pBluescript (Stratagene). pTX0375 was constructed by cloning a 2.5kb *Spe*I fragment from pTX0374 into *Spe*I-digested pPS1128. This plasmid was constructed in two stages. In the first, the left hand *Eco*RI site of pPS971 (Weedon *et al*, Int. J. Cancer, in press) was converted to a *Swa*I site to create pPS115. In the second, the 350bp *Spe*I-*Afl*III fragment of pPS115 was replaced with a linker prepared by

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annealing the two oligonucleotides:

5'- CTAGTATCGATTGTTAATTAAGGGCGTGGCC (SEQ ID NO:~~19~~ 18) -3' and

5'- TTAAGGCCACGCCCTTAATTAACAATCGATA (SEQ ID NO:~~20~~ 19) -3'.

pPS1022 was constructed from pPS972 by conversion of the right hand *EcoRI* site to a *SwaI* site.